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94-08729

Utility of Methylene Blue for the Reversal of
Excessive Levels of Methemoglobin

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ABSTRACT

Many new prophylactic and therapeutic compounds are being studied as potential sources of methemoglobin useful in counteracting the lethal effects of cyanide intoxication. The formation of methemoglobin also leads to a reduction in the blood oxygen carrying capacity which may, in extreme cases, lead to lethal consequences. The i.v. administration of Methylene blue rapidly reverses methemoglobin to hemoglobin. Unanticipated high levels of methemoglobin (65 - 85%) in three sheep exposed to propiophenone derivatives led to a lethal outcome in one untreated sheep and complete recovery in two sheep which were treated with 3.0 mg/kg methylene blue i.v. Methemoglobin was reduced to safe levels within minutes following administration. A similar exposure of dogs to propiophenone derivatives led to high levels (77 - 78%) of methemoglobin which were readily reversed following the intravenous administration of the same dose of methylene blue.

Following cyanide intoxication in an anesthetized dog model and treatment with NaNO_2 (or other methemoglobin formers) high levels of methemoglobin (20-50%) remain which are in excess of that required to bind the cyanide ion. The excessive levels of methemoglobin can be rapidly reversed through the intravenous infusion of methylene blue without re-inducing cyanide toxicity. The adjunctive infusion of methylene blue following effective immediate first aid therapy with sufficient doses of a potent methemoglobin former will lead to more rapid restoration of blood oxygenation and recovery of the casualty.

Introduction

The rapid formation of methemoglobin is often used as a technique to counteract cyanide intoxication. The creation of minimal levels of methemoglobin are also used to serve as a pretreatment to prevent cyanide intoxication (1).

Low levels of methemoglobin are non-toxic and do not create a performance decrement. As levels of methemoglobin increase, the total oxygen-carrying capacity is reduced. If methemoglobin levels increase to sufficiently high levels, a state of hypoxia ensues(2) which may lead to fatal consequences.

Methemoglobin can be reversed rapidly by the intravenous infusion of methylene blue(3). The reversal of methemoglobin with methylene blue is followed by an increase in total oxygen-carrying capacity and recovery of the animals.

In the treatment of cyanide intoxication, it is desirable to create sudden high levels of methemoglobin which will rapidly interact with the circulating cyanide ion to remove the cyanide from the cytochrome oxidase. When the cyanide ion has been removed by methemoglobin to form cyanomethemoglobin, the intoxicated animal will recover (4).

During recovery from cyanide intoxication the excess methemoglobin which is no longer required for cyanide detoxification is not advantageous to the animal, and, in fact, creates a reduction in oxygen-carrying capacity. The rapid reversal of the excess methemoglobin to hemoglobin and an increase in oxygen-carrying capacity will be most beneficial to the intoxicated animal.

The reversal of methemoglobin to hemoglobin can also take place in the presence of cyanide without an induction or a re-introduction of cyanide intoxication. These observations may suggest that methylene blue may be used as "follow on" therapy to the Sodium Nitrite first aid therapy for cyanide intoxication.

Methods

Four unanesthetized male beagle dogs were given an oral dose of a PEG solution of p-Hydroxylaminoheptanophenone (PAHP-OH) by oral gavage equivalent to 15 mg/kg. Two of these animals developed high (78%) concentrations of methemoglobin and were treated intravenously with 3.5 mg/Kg of methylene blue. The two remaining animals formed moderate (38 to 66%) levels of methemoglobin and were not treated with methylene blue. Heparinized venous blood samples were collected and monitored immediately for the

formation of methemoglobin and the corresponding reduction in oxyhemoglobin activity. Methemoglobin and oxyhemoglobin were determined with a Radiometer OSM-3 Hemoximeter immediately after the venous sample was obtained.

Three unanesthetized sheep were treated intravenously with 12 mg/kg p-Aminooctanophenone (PAOP) leading to the formation of 65%, 87% and 90% methemoglobin. The animal which achieved 90% methemoglobin died without therapy. The remaining two sheep were treated intravenously with 3.0 mg/kg methylene blue. Venous blood samples were collected and monitored for the formation of methemoglobin and oxyhemoglobin.

Solutions of methylene blue were developed in saline at a concentration of 10 mg/ml. Methylene blue solution was administered by intravenous infusion at a dose of 3.0 mg/kg in sheep and 3.5 mg/kg in dogs.

Solutions of sodium cyanide were developed in saline at a concentration of 4.0 mg/ml and administered as a i.v. infusion or at a concentration of 10 mg/ml for the acute cyanide intoxication (LD_{100}) dose of 2.5 mg/Kg by bolus i.v. injection.

Cyanide was infused in three anesthetized animal experiments at a rate of 7 mg/minute until respiratory arrest occurred. The cyanide infusion was halted and therapy was administered 30 minutes after the infusion of cyanide was halted. Acute cyanide intoxication utilized a bolus i.v. injection of 2.5 mg/Kg sodium cyanide which was followed by therapy 2.0 minutes after initiation of the i.v. injection.

The "NAP" antidotal combination of sodium nitrite (20 mg/Kg), Atropine sulfate (1 mg/Kg) and 2-PAM (25 mg/Kg) was administered intramuscularly. The animals were allowed to form methemoglobin and to recover for 40 minutes before the intravenous infusion of 3.5 mg/Kg methylene blue to reverse methemoglobin to hemoglobin. No additional form of therapy was presented to the animals.

Additional studies were carried out in pentobarbital anesthetized (32 mg/Kg) beagle dogs to monitor the physiological course of cyanide intoxication by intravenous infusion and recovery in the presence of sodium nitrite and methylene blue therapy. Both jugular veins were catheterized for the infusion of sodium cyanide solution and methylene blue as well as the collection of blood samples for the recording of methemoglobin and oxyhemoglobin activity. The animals were intubated and respiratory activity was monitored with a pneumotachometer.

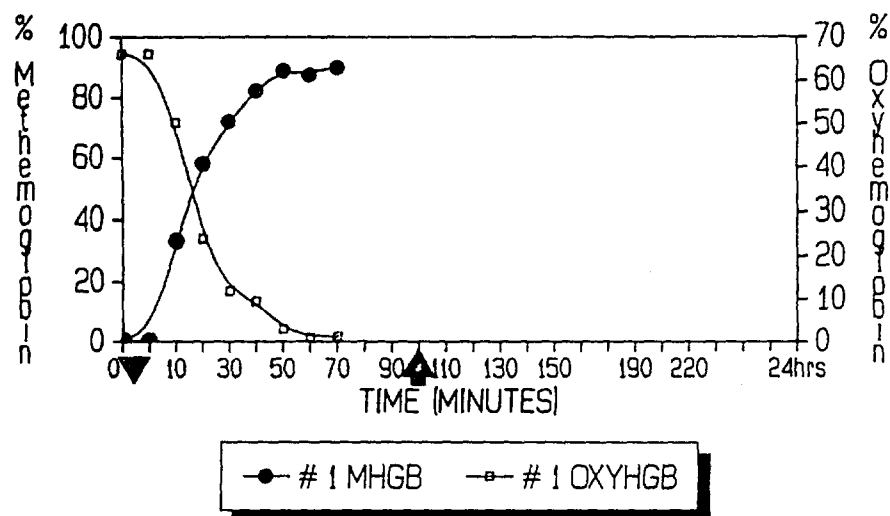
Results

Figure #1a. illustrates the formation of a high level of methemoglobin (solid circles) in one sheep following the intravenous infusion of 12 mg/kg PAOP.

The formation of methemoglobin leads to a corresponding decrease in oxyhemoglobin (open squares) levels.

The low level of oxyhemoglobin resulted in death of the animal.

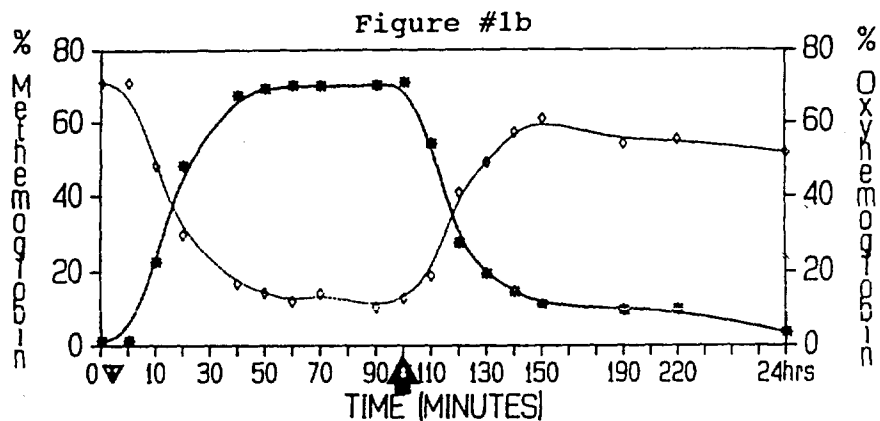
Figure #1a



#1 animal died prior to therapy
 ▼ PAOP, 12 mg/Kg I.V.
 ▲ METHYLENE BLUE, 3 mg/Kg I.V.

Two sheep were treated with 3.0 mg/kg methylene blue immediately after the formation of high levels of methemoglobin and the severe reduction in oxyhemoglobin concentration.

Figure #1b illustrates the methemoglobin and oxyhemoglobin of one of these two animals methemoglobin intoxicated animal. In this animal the intravenous infusion of 3.5 mg/Kg of methylene blue induced the rapid reduction of methemoglobin to less than 10% and the corresponding oxyhemoglobin increased to near pre-exposure levels. The animals recovered a short time after the administration of the methylene blue.

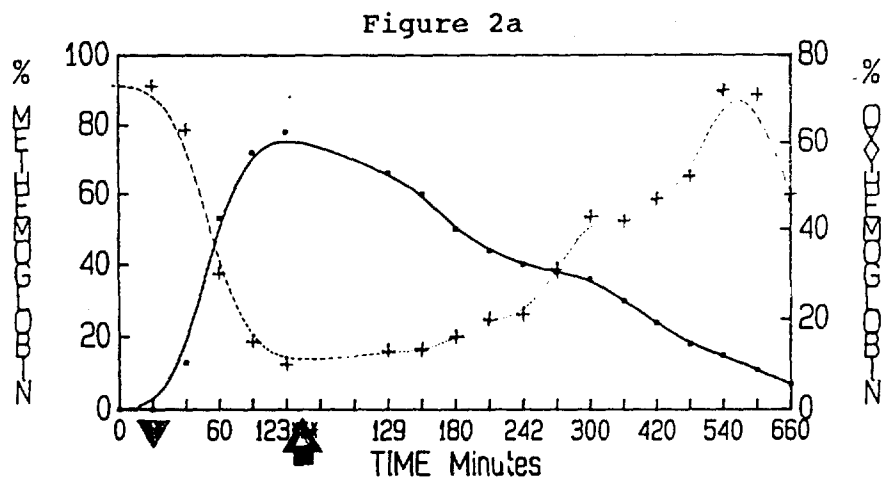


▼ PAOP, 12 mg/Kg I.V.
 ▲ METHYLENE BLUE, 3 mg/Kg I.V.

—●— # 3 MHGB -○- # 3 OXYHGB

In 4 dogs treated with 15 mg/kg of PAHP-OH, two of the animals lapsed into a period of severe methemoglobin formation with a corresponding reduction in oxyhemoglobin requiring immediate intravenous therapy with methylene blue.

A graph of one of the dogs with high levels of methemoglobin is shown in Figure 2a, the methemoglobin was rapidly reversed to hemoglobin and the oxyhemoglobin rapidly recovered.



▼ PAHP-OH, 15mg/kg
 ▲ Methylene blue, 3.5 mg/kg, I.V.

—●— MHGB -+- OXYHGB 1

Two additional animals not as severely intoxicated from high levels of methemoglobin were allowed to recover without therapy. The normal rate of recovery from elevated levels of methemoglobin of one of these dogs is shown in Figure 2b. The high levels of methemoglobin were reduced at a relatively low rate during an 8 hour observation period. During the same period of time, the oxyhemoglobin recovery was significantly lower than occurred in animals which had been treated with methylene blue. Although this animal recovered completely, the recovery of critical oxyhemoglobin required a significantly longer period of time than animals which suffered higher levels of methemoglobin and were treated with methylene blue.

Figure 2b

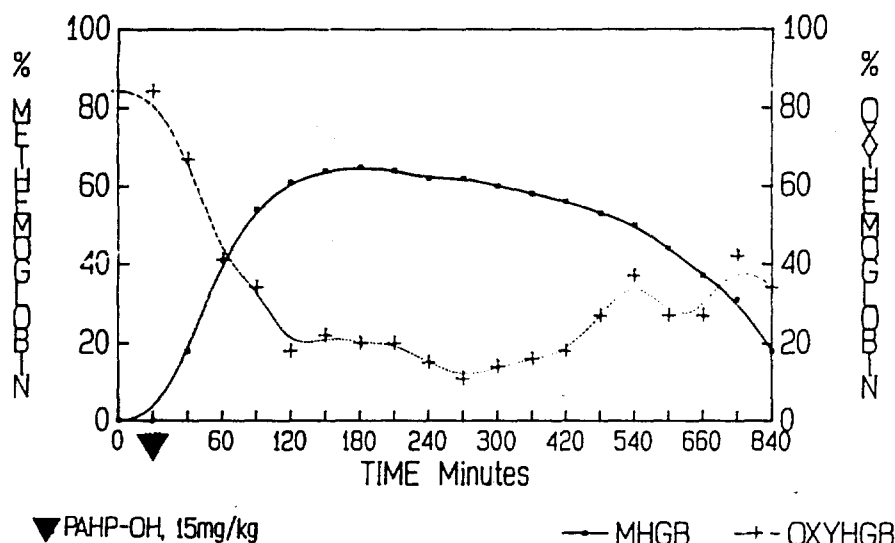


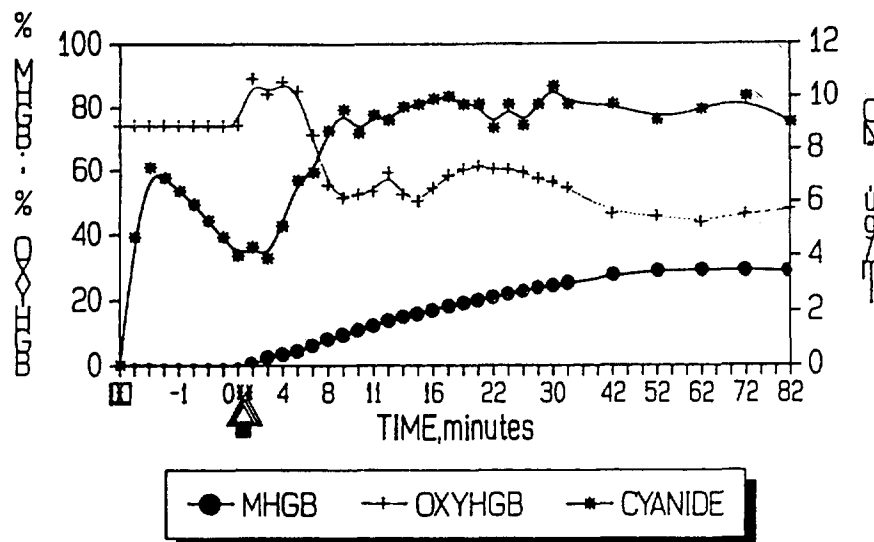
Figure 3a illustrates the formation of methemoglobin by the "NAP" combination in the presence of an acute exposure to cyanide.

The formation of methemoglobin (solid circles) reversed cyanide intoxication and maintained the cyanide (*) (bound to methemoglobin) at a steady level for the remainder of the observation period.

In a similar manner, the oxyhemoglobin (+) remained depressed for the duration of the period during which methemoglobin was elevated.

Figure 3a

EFFECT OF SODIUM NITRITE, ATROPINE & 2pam
(NAP) I.M. ON METHEMOGLOBIN AND
OXYHEMOGLOBIN AFTER EXPOSURE TO CYANIDE



[] Cyanide-2.5 mg/kg, i.v.
 [] [] Sodium Nitrite-20 mg/kg.
 [] [] Atropine-1 mg/kg, [] [] 2pam, 25mg/kg.

Figure 3b demonstrates a similar phenomena of acute cyanide intoxication treated with "NAP" to form methemoglobin leading to a reversal of cyanide intoxication and constant methemoglobin values.

After the successful formation of methemoglobin (solid circles) to treat the cyanide intoxication, the excess methemoglobin was reversed by the intravenous infusion of methylene blue. Reversal of methemoglobin is accompanied by a corresponding increase in oxyhemoglobin(+).

The blood cyanide (*) concentration increased to a constant level and this concentration was maintained during the observation period even after the administration of 3.5 mg/kg methylene blue.

In a total of 3 animals exposed to an acute dose of sodium cyanide, treated immediately with a rapid methemoglobin former and provided with the methylene blue as "follow on therapy" the animals recovered completely without any signs of cyanide re-intoxication.

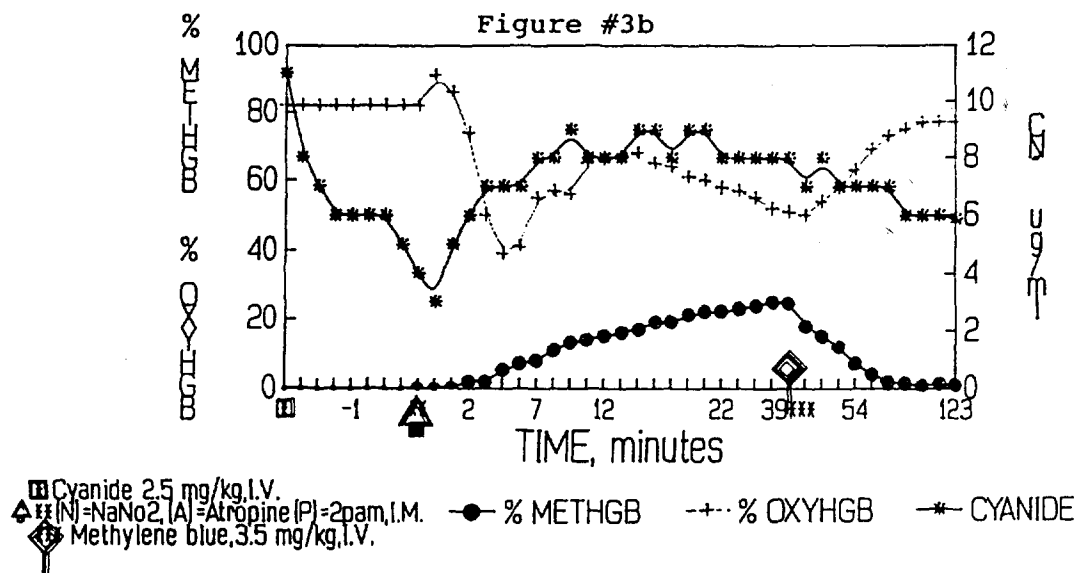
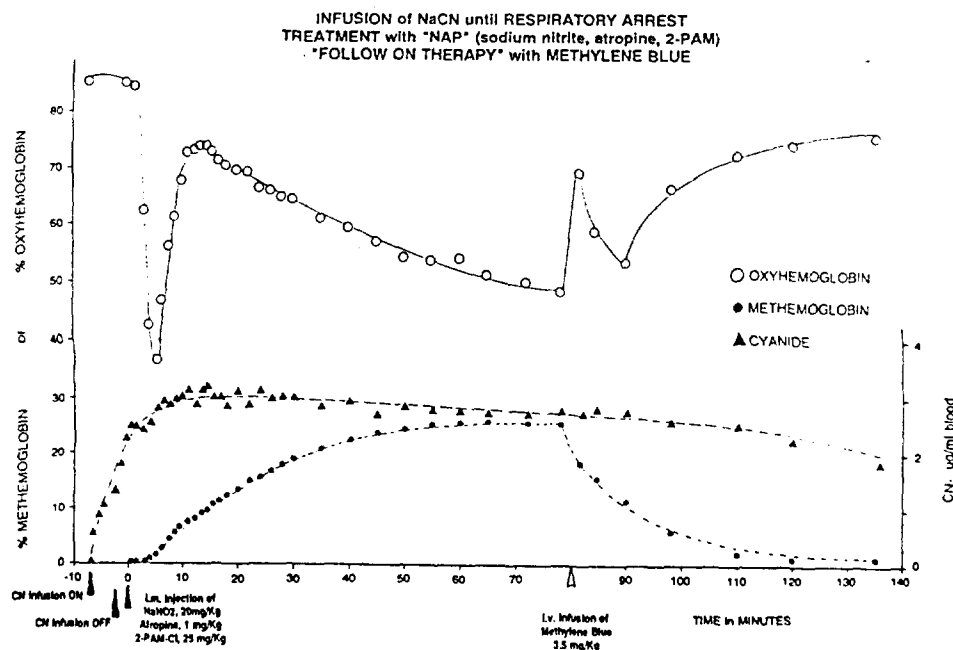


Figure #4 demonstrates recovery of an anesthetized beagle dog from a slow intravenous infusion of sodium cyanide sufficient to cause respiratory arrest which required treatment with a methemoglobin forming combination. Soon after recovery of respiration, "follow on" therapy with methylene blue demonstrated a reduction in methemoglobin and a recovery of oxyhemoglobin. The administration of methylene blue did not reinduce the cyanide intoxication in a total of 4 animal.



Discussion

The use of a methemoglobin-forming compound to treat cyanide intoxication or to prevent cyanide intoxication may lead to the formation of unexpectedly high levels of methemoglobin. The formation of unexpectedly high levels of methemoglobin will lead to a corresponding decrease in oxyhemoglobin. As shown in these animal experiments, when methemoglobin is reduced suddenly by the infusion of methylene blue, the oxyhemoglobin rapidly recovers.

Methylene blue is a water soluble compound which is easily administered by intravenous infusion. Low concentrations of methylene blue readily reverse methemoglobin. In-vitro high levels of methylene blue induce methemoglobin, therefore, methylene blue has been suggested as a source of methemoglobin to treat cyanide intoxication.

Since methylene blue can be used to reverse excessive levels of methemoglobin, it leads to the possibility that methylene blue may be considered as a "follow on" therapy for casualties of cyanide intoxication which have been treated by the administration of a methemoglobin former, such as sodium nitrite. This makes it possible to treat cyanide intoxication aggressively with a rapid acting methemoglobin former and then to rapidly reverse the excessive levels of methemoglobin with methylene blue.

During the "follow on" therapy with methylene blue for cyanide intoxication which has been treated with methemoglobin formers, most of the methemoglobin will be reduced to hemoglobin, however, a small quantity of methemoglobin will remain to keep the cyanide ion bound to prevent re-intoxication by cyanide. This possibility may be suggested by the constant level of blood cyanide during the reversal of the methemoglobin. If cyanide ion were liberated during the methemoglobin reversal, the cyanide ion would have disappeared from the red cell and diffused into tissues to allow a re-intoxication by the liberated cyanide. In the acute cyanide intoxication and during cyanide infusion the whole blood cyanide levels remained constant before and after therapy with methylene blue.

CONCLUSION

Excessive levels of methemoglobin resulting from treatment or pretreatment of cyanide intoxication with methemoglobin formers can readily be reversed with methylene blue.

Methylene blue provides effective "follow on" therapy for cyanide treatment with methemoglobin formers in order to rapidly restore the oxyhemoglobin without resulting in a re-intoxication of the animals by cyanide.

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